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## WHAT IS CLAIMED IS:

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- 1. A process for the sequence analysis of a formed or forming polypeptide which comprises the steps of producing a reaction mixture containing a peptide ladder comprising a series of adjacent polypeptides in which each member of the series differs from the next adjacent member by one amino acid residue and thereafter determining the differences in molecular mass between adjacent members of the series by mass spectroscopy, such differences coupled with the positions of said adjacent members in the series being indicative of the identity and position of the said amino acid residue in the formed or forming peptide.
- 2. The process of claim 1 wherein a plurality of peptide ladders are produced from separate formed polypeptides in the same reaction zone.
  - 3. The process of claim 1 wherein a plurality of peptide ladders are produced from separate formed polypeptides in separate reaction zones.
- 4. The process of claim 2 or 3 wherein the polypeptide is absorbed on a membrane support.

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- 5. The process of claim 1 wherein the formed polypeptide is a modified polypeptide.
- 6. The process of claim 5 wheren the polypeptide is phosphorylated.
- 7. The process of claim 5 wherein the polypeptide includes a phosphorylated serine residue.

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8. A process for the sequence analysis of a formed polypeptide which comprises the steps of:

a: reacting the polypeptide with a molar excess of a pair of reagents comprising a coupling reagent and a terminating reagent each of which forms a reaction product with a terminal amino acid residue of the polypeptide to be analyzed under the same reaction conditions; the reaction product formed between the terminating reagent and the terminal amino acid residue of the polypeptide being stable under all subsequent reaction conditions; the reaction product formed between the coupling reagent and terminal amino acid residue of the polypeptide to be analyzed being removable as a cleavage product from the original polypeptide together with the terminal amino acid to which it is attached by changing the reaction conditions;

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b: changing the reaction conditions so that the cleavage product separates, thereby to form a reaction mixture comprising:

- i. unreacted coupling and terminating reagents.
- ii. a first reaction product which is the reaction product between the original polypeptide and the terminating reagent,
  iii. a newly formed polypeptide from which the terminal amino acid residue has been removed;
  c: repeating steps <u>a</u> and <u>b</u> any selected number of cycles thereby to form a final mixture which comprises:
  - i. reaction product between the original polypeptide and the terminating reagent, ii. a peptide ladder which is series of adjacent reaction products each member of which is formed by reaction between the terminating reagent and the terminal amino acid residue of a fraction of the newly formed polypeptide of each cycle, the number of such reaction products, including said first reaction product, being equal to the number of cycles conducted; and

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d: determining the differences in molecular mass between adjacent members of the series of reaction products by mass spectroscopy, such differences being equal to the molecular mass of the amino acid residue cleaved from the original polypeptide and from each subsequent polypeptide of the series, such differences coupled with the positions of said adjacent members in the mass spectrum being indicative of the identity and position of that amino acid residue in the original polypeptide.

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9. The process of claim 8 wherein the coupling and terminating reagents react with the terminal amino acid at the amino terminal of the original polypeptide.

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10. The process of claim 9 wherein the coupling reagent is phenyl isothiocyanate and the terminating reagent is phenyl isocyanate.

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11. The process of claim 8 wherein the coupling and terminating reagents react with the terminal amino acid at the carboxy end of the original polypeptide.

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12. A process as in claim 8, 9, 10 or 11 wherein at least two different polypeptides are simultaneously analyzed in the same reaction mixture.

- 13. The process of claim 8, 9, 10 or 11 wherein a plurality of peptide ladders are produced from separate formed polypeptides in the same reaction zone.
- 14. The process of claim 8, 9, 10 or 11 wherein a plurality of peptide ladders are produced from separate formed polypeptides in separate reaction zones.

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- 15. The process of claim 13 wherein the polypeptide is abosrbed on a membrane support.
- 16. The process of claim 14 wherein the polypeptides are absorbed on resin supports.
- 17. The process of claim 8, 9, 10 or 11 wherein the formed polypeptide is a modified polypeptide.
- 18. The process of claim 8, 9, 10 or 11 wherein the formed polypeptide is a modified polypeptide which is modified by phosphorylation.
- 19. The process of claim 8, 9, 10 or 11 wherein the formed polypeptide is a modified polypeptide which is modified by the presence of a phosphorylated serine residue.

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20. A process for the sequence analysis of a formed polypeptide which comprises the steps of:

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a: reacting the polypeptide with a coupling reagent under conditions such that the terminal amino acid residue of only a portion of the polypeptide to be analyzed reacts with the coupling reagent, the reaction product formed between the coupling reagent and the terminal amino acid of the polypeptide to be analyzed being removable as a cleavage product from the original polypeptide together with the terminal amino acid to which it is attached by changing reaction conditions; b: changing the reaction conditions so that the cleavage product separates, thereby to form a reaction mixture comprising:

- i. unreacted coupling agent
- ii. the cleavage product
- iii. unreacted original formed polypeptide
- iv. a newly formed polypeptide with one less
  amino acid residue than the original
  polypeptide
- c: repeating steps <u>a</u> and <u>b</u> any selected number of cycles thereby to form a final mixture which comprises a series of adjacent polypeptides adjacent members of which differ by one amino acid residue; and

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- d: determining the differences in molecular mass between adjacent members of the series of mass spectroscopy, such differences being equal to the mass of the amino acid residue cleaved from the original polypeptide and from each subsequently formed polypeptide of the series, such differences coupled with the position of said adjacent members in the mass spectrum being indicative of the identity and position of that amino acid residue in the original polypeptide.
- 21. The process of claim 20 wherein the coupling reagent reacts with the terminal amino acid at the amino terminal of the original polypeptide.
- 22. The process of claim 21 wherein the coupling reagent is phenyl isothiocyanate.
- 23. The process of claim 20 wherein the coupling reagent reacts with the terminal amino acid at the carboxy end of the original polypeptide.
- 24. The process of claim 20, 21, 22 or 23 wherein at least two different polypeptides are simultaneously analyzed in the same reaction mixture.

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- 25. The process of claim 20, 21, 22, or 23 wherein a plurality of peptide ladders are produced from separate formed polypeptides in the same reaction zone.
- 26. The process of claim 20, 21, 22, or 23 wherein a plurality of peptide ladders are produced from separate formed polypeptides in separate reaction zones.
- 27. The process of claim 25 wherein the polypeptide is absorbed on a membrane support.
- 28. The process of claim 26 wherein the polypeptides are absorbed on resin supports.
  - 29. The process of claim 20, 21, 22 or 23 wherein the formed polypeptide is a modified polypeptide.
  - 30. The process of claim 20, 21, 22 or 23 wherein the formed polypeptide is a modified polypeptide which is modified by phosphorylation.
  - 31. The process of claim 20, 21, 22 or 23 wherein the formed polypeptide is a modified polypeptide which is modified by the presence of a phosphorylated serine residue.

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A process for the sequence analysis of a forming polypeptide which is being formed by cyclical, coupling and deblocking of N < -blocked amino acid residues to form a final polypeptide one terminal of which is bound to a support which process comprises collecting a support bound sample after each cycle, mixing the collected samples, cleaving from the support in the collected samples, the polypeptides formed thereon to produce a reaction mixture containing a peptide ladder comprising a series of adjacent polypeptides in which each member of the series differs from the next adjacent member by one amino acid residue and thereafter determining the differences in molecular mass between adjacent members of the series by mass spectroscopy, such differences coupled with the positions of said adjacent members in the series being indicative of the identity and position of the said amino acid residue in the formed or forming peptide.

33. A process for the sequence analysis of a forming polypeptide which is being formed by cyclical coupling and deblocking of N <-blocked amino acid residues to form a final polypeptide one terminal of which is bound to a support which process comprises:

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- a. Conducting the coupling step of each cycle with a mixture of the same amino acid residue the major portion of which is blocked with a blocking group removable under selected reaction conditions, the minor portion of which is blocked with a blocking group which is stable under the said reaction conditions,
- b. Conducting each deblocking step of each cycle under conditions such that the removable blocking group is removed,
- c. Repeating steps a and b, and
- d. Removing the products from the support to obtain a mixture containing a peptide ladder comprising a series of adjacent polypeptides in which each member of the series differs from the next adjacent member by one amino acid residue and thereafter determining the differences in molecular mass between adjacent members of the series by mass spectroscopy, such differences coupled with the positions of said adjacent members in the series being indicative of the identity and position of the said amino acid residue in the formed or forming peptide